

defined. In the Kyte and Doolittle scale<sup>11</sup>, proline is considered a slightly hydrophilic amino acid, however the structural characteristic of the side chain of proline should impart a more hydrophobic character. This is reflected in the Akamatsu and Fujita scale<sup>12</sup>,  
5 where the hydrophobic value is close to other hydrophobic amino acids, exactly between alanine and methionine.

Two different complementary peptides, reflective of these two possible hydrophobic characteristics of proline, were synthesized. A slightly hydrophilic proline is best complemented by  
10 alanine, so the sequence ASA was chosen. A hydrophobic proline is best genetically complemented by arginine, and RTR was chosen. To increase the potential affinity for N-acetyl-PGP, complementary  
peptides were synthesized in multimeric forms, starting from a polylysine core, and spaced from the core with two glycines. The  
15 simple linear RTR and RTRGG sequences were also synthesized to verify the specificity of the RTR sequence in the multimeric peptides (Figure 1).

### EXAMPLE 3

#### Peptide Synthesis and Isolation

Complementary peptides were synthesized using Solid  
5 Phase Peptide synthesis following Fmoc methodology on a 9050  
Peptide synthesizer from Perseptive Biosystem. The linear peptides  
were synthesized using an Amide-polyethylene glycol graft  
polystyrene (PEG-PS) resin and *O*-pentafluorophenyl ester pre-  
activated amino acids. The branched peptides were synthesized  
10 starting from a Fmoc-Alanine-PEG-PS resin, with either one or two  
coupling cycles with Fmoc-K-Fmoc-OH activated with HATU/DIPEA.  
The following couplings were achieved using Fmoc -amino acids  
activated with HATU/DIPEA. The Fmoc deprotection reagent was 1%  
DBU, 1% Piperidine in dimethylformamide. The peptides were  
15 cleaved from the resins by adding 10 ml of trifluoroacetic acid  
(TFA)/phenol/thioanisole/H<sub>2</sub>O/ethanedithiol 93/2/2/2/1 and  
incubated at room temperature for 5 hours. The mixtures were  
filtered and the peptides precipitated in cold ethyl ether. The  
precipitates were collected and solubilized in H<sub>2</sub>O for lyophilization.

All peptides were purified by reverse phase high performance liquid chromatography (RP-HPLC), using a Dynamax RP C18 (300x10mm i.d.), and equilibrated at 3 ml/min using a linear gradient from 5% CH<sub>3</sub>CN to 60% CH<sub>3</sub>CN in 0.1% TFA in 40 minutes. The fractions  
5 containing the peptide were acidified with 1 N HCl to help in the elimination of TFA, and lyophilized. Peptide identity was confirmed by time of flight matrix assisted laser desorption ionization mass spectroscopy. Purity was confirmed by analytical RP-HPLC.

For large-scale synthesis of N-acetyl-PGP, an alternative  
10 method was used to increase the yield of the product. In this method, the dipeptide *t*-Boc-PG was coupled to Pro-Merrifield resin using the dicyclohexylcarbodiimide/1-hydroxybenzotriazole  
procedure. After the removal of the N-terminal protection and acetylation using acetic anhydride, the peptide was cleaved from the  
15 resin using anhydrous hydrofluoric acid. The product was purified on a silica gel column using chloroform: methanol (90:10 v/v) as the eluent. Homogeneity was confirmed by RP-HPLC on a Vydac C18-analytical column equilibrated at a flow rate of 1.2 ml/min and eluted with a linear gradient from 0% to 30% acetonitrile in water